Abstract

This work presents research findings on the α-helix within diverse biomolecular settings. Initially, we examined alanine-rich peptides, which readily adopt an α-helix structure in aqueous environments. Employing both calorimetric (DSC) and CD spectroscopy techniques, we analysed the system. Calorimetrically, we obtained precise data on the temperature-dependent heat capacities of alanine peptides of varying lengths. By applying the statistical-thermodynamic Lifson-Roig model of helix-coil transition, we effectively characterized the experimental data, acquiring fundamental parameters for the folding of the alanine α-helix. Despite the intricacies of thermal data analysis, we accurately determined these parameters. Notably, we determined the nucleation constant calorimetrically, which present a novel achievement. Spectroscopic analysis of thermal denaturation data agrees well with calorimetric findings. We demonstrated the inadequacy of the current method for interpreting CD signals of helical peptides, which marginally underestimates helicity of the peptides. Therefore, we developed a new computational approach for CD signal analysis, yielding a more precise estimation of helicity changes during peptide unfolding. This model enhances CD data analysis, extracting more information from experimental data with increased parameter reliability. Computer program for estimation of helicity is freely available on GitHub (https://github.com/rubiscoo/Dichroic-CD-model).

In the latter part of our study, we investigated α-helices within intrinsically disordered proteins (IDPs). Utilizing bioinformatic analysis, we explored helical binding motifs prevalent in IDPs, which play a crucial role in binding to target proteins. Sequence analysis elucidated residual structure within sequences, emphasizing the abundance of amino acid residues with heightened helical propensities. Notably, leucine's prevalent occurrence in these motifs plays a dual role, stabilizing residual structures and enhancing interactions with target proteins, as confirmed by spectroscopic (CD) and calorimetric (ITC) experiments. Additionally, we investigated toxin-antitoxin interactions, where the antitoxin, upon binding the toxin, forms an α-helix. Employing the Lifson-Roig model of helix-coil transition and thermodynamic dissection, we distinguished thermodynamic contributions of binding and folding, elucidating the origins of high-affinity interaction and revealing exceptional interaction optimization comparable to high-affinity interaction among globular proteins. Furthermore, our research yields valuable α-helix folding parameters for sequences with low helical propensities.